Importance of Oxidative Damage on the Electron Transport Chain for the Rational Use of Mitochondria-Targeted Antioxidants

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Abstract: The electron transport chain (ETC) has become a promising pharmacological target as ETC impairment by reactive oxygen species (ROS) has been detected in several diseases. Therefore, for a better understanding of the actions of mitochondria-targeted antioxidants, it must be considered the interplay between the sources of ROS during disease, the chemical interconversions of ROS and their differential reactivity with ETC components. This review contrasts these aspects with available data about mitochondrial damage in specific diseases to give an insight into the importance of ROS chemistry in the rational use of mitochondria-targeted antioxidants, putting emphasis on the case of MitoQ.

Keywords: Lipid peroxidation, thiol oxidation, iron, respiratory chain, MitoQ, ischemia-reperfusion, neurodegeneration, liver diseases.

1. INTRODUCTION

Mitochondria are complexes organelles that carry out a vast number of functions, including transduction of energy from the oxidation of metabolic fuels to generate ATP through the establishment of a transmembrane potential, regulation of cellular calcium concentration [1], iron transport and storage for the synthesis of heme and iron-sulfur clusters [2], compartmentalization of metabolic pathways, readjustment of carbon and nitrogen metabolism [3, 4], regulation of antioxidant synthesis through retrograde regulation of nuclear genes [5], and modulation of cell cycle progression and cell death [6, 7, 8], among others. Thus, this organelle is now considered to be a machine operating near its maximal capacity and extremely prone to damage, often with disastrous consequences for cells [9]. This idea is reinforced by the fact that mitochondrial dysfunction is involved in almost all diseases and pathological processes [10].

Diseases involving mitochondrial dysfunctions have been classified in four broad categories: 1) diseases provoked by mutations in mtDNA, 2) diseases caused by mutations in nuclear DNA leading to altered regulatory processes and toxic effects in mitochondria due to mutated proteins, 3) altered mitochondrial function in cancer cells, and 4) diseases related to acute damage to mitochondria due to ischemia, inflammation and intoxication [10]. Some examples of the latter class of mitochondrial disorders will be the focus of this review, emphasizing some of the mechanistic aspects of damage to electron transport chain (ETC) by reactive oxygen species (ROS) and summarizing some aspects regarding the use of mitochondria-targeted antioxidants to mitigate the damage to the ETC, taking the case of MitoQ as a paradigm.

2. OXIDATIVE DAMAGE TO ETC DURING DISEASE

A significant number of mitochondrial functions depend on the integrity of the ETC, which is made up of an assembly of four multimeric enzymes embedded in the inner mitochondrial membrane. The ETC oxidizes reducing equivalents produced by the oxidation of metabolic fuels and transports electrons to a final acceptor (i.e., oxygen). Three of these four complexes act as redox pumps, coupling electron transport to vectorial proton translocation from the matrix to intermembrane space and generating an electrochemical gradient across the inner membrane that is used for ATP generation by F_1F_0 ATP synthase, ionic transport, and protein import. Electron transfer at ETC enzymes is carried out by several prosthetic groups, such as iron-sulfur clusters (Fe-S) and cytochromes *b*, *c*, and *a*, attached to apoproteins by covalent and non-covalent interactions. The transport of electrons between these complexes is mediated by soluble cytochrome *c* and the lipophilic carrier ubiquinone [1]. These carriers also have other important functions unrelated to electron transfer; for example, cytochrome *c* is an important mediator of apoptosis, while ubiquinone is a potent lipophilic antioxidant whose absence can compromise the integrity of the cell during oxidative challenges [11].

The mitochondrial ETC constitutes one of the most important cellular targets of ROS. During pathological processes, the ROS attacking mitochondrial complexes can be produced by enzymatic extramitochondrial sources, by disruption of electron flux through ETC complexes or by

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mitochondrial overload with transition metals that catalyze ROS generation (e.g., iron). It has been proposed that the most important source of cellular ROS is the family of NADPH oxidase enzymes (Nox) [12, 13]. Mitochondria have also been considered to be the main source of ROS in cells [14, 15], although this notion has been questioned because in isolated mitochondria and intact cells, the production of ROS may be exacerbated and may not represent the *in vivo* ROS due to the damage inflicted by isolation procedures and high glucose concentrations required to ROS detection, respectively [16]. In addition, it has been argued that the quantity of ROS produced by mitochondria is negligible when is compared with the ROS production by activated Nox enzymes [16]. However, these opposing views have been conciliated by recent reports about the localization of Nox4 in mitochondria and its role in ROS generation in renal, cardiac and breast cells during the pathogenesis of diabetes [17], cardiac dysfunction [18] and cancer [19], respectively. Furthermore, it has been shown an interplay between the ROS produced in mitochondria and the generated by Nox in hypertensive vasculature through a positive feedback loop in which, the peroxynitrite radical ($NOO^{\bullet}$) produced by the activation of Nox by angiotensin II and the activity of the nitric oxide synthase, causes damage to ETC function and enhances mitochondrial H_2O_2 generation, which in turn, further activates Nox and leads to sustained $O_2^{\bullet-}$ production and decreased NO bioavailability [20, 21].

Likewise, during allergic inflammation in human airways epithelial cells, it has been observed that Nox activity from pollen particles induces oxidative damage in the core II subunit of complex III and stimulates sustained production of H_2O_2, leading to an increase in some markers of airway inflammation. Thus, it was established a relationship between Nox enzymes, mitochondrial dysfunction and exacerbated symptoms during allergic airway inflammation [22, 23]. In agreement with these findings, the exposure to particulate matter air pollution <2.5 μm in diameter stimulates the production of ROS in the quinol oxidase (Q_O) site of complex III, which can contribute to alveolar epithelial dysfunction, lung injury and inflammation [24].

As mentioned above, the oxidative damage to the ETC can be auto-induced by the disruption of electron flux through ETC complexes, leading to increased electron leakage and partial reduction of oxygen to generate ROS. One of the best characterized examples of this mechanism of ETC damage occurs during the heart damage by ischemia-reperfusion. During ischemia, occurs both a decrease in oxygen concentration and a depletion in ADP that causes accumulation of one-electron donors (i.e. ubisemiquinone radical, $QH^{\bullet}$). Upon reestablishment of oxygen by reperfusion, there is a burst of ROS production during the first minutes of reoxygenation because $QH^{\bullet}$ radicals react directly with O_2 to generate $O_2^{\bullet-}$, which is converted to H_2O_2 by Mn-SOD [25, 26]. This burst of ROS inhibits the activities of the complexes I, III and IV due to cardiolipin peroxidation and ubiquinone loss (Fig (**1**), Table **1**) [26, 27, 28]. The disruption in ETC function leads to compromised membrane potential and diminished ATP synthesis, causing an alteration in cellular Ca^{2+} and Na^{+} homeostasis due to decrease in the activity of ATP-dependent ion pumps and opening of mitochondrial permeability transition pores with potentially catastrophic consequences for the cell [29]. Another example in which the disruption of electron transport induces ROS overproduction and development of disease is the infection with hepatitis C virus. It has been observed that the core protein of this virus can interact with the outer membrane of mitochondria, which both augments Ca^{2+} uptake and depletes the pool of reduced glutathione, which in turn leads to complex I inhibition and ROS production (Table **1**) [30].

Iron can exert deleterious effects on almost all biomolecules by catalyzing the formation of highly toxic ROS, such as hydroxyl radical ($OH^{\bullet}$) or iron-centered radicals such as ferryl and perferryl [31, 32]. Abnormal accumulation of iron and mitochondrial dysfunction is a common feature of some brain diseases, such as Parkinson's disease [33, 34, 35] or Alzheimer's disease [36, 37] (Table

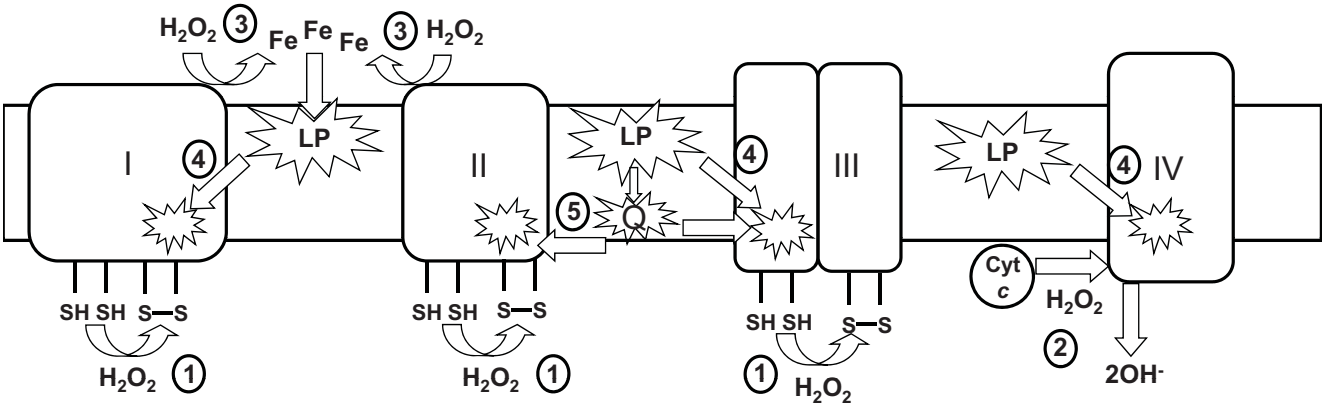

Fig. (1). Overview of the effects of iron (Fe), H_2O_2, lipoperoxidation (LP) and thiol (-SH) oxidation on ETC function. (1) H_2O_2 inhibits the activity of complexes I, II and III through the oxidation of vicinal thiols to form internal disulfides (S-S). (2) Complex IV reduces H_2O_2 using reduced cytochrome *c* as an electron donor. (3) H_2O_2 also can release iron from mitochondrial pools (i.e. from cytochromes or iron-sulfur clusters). (4) Iron overload induces lipoperoxidation, which impairs directly the activity of all complexes except complex II, as this complex does not depend on the phospholipid cardiolipin to achieve its function [43] and a lipophillic antioxidant does not protect complex II activity from lipoperoxidative conditions [47]. (5) Lipoperoxidation also depletes ubiquinone (Q) and impairs the activity of complexes I, II and III.

Table 1. Diseases Involving Mitochondrial Dysfunctions by Oxidative Insults and Therapeutic Benefits of MitoQ. (+): Protective Effect; (-): None Protective Effect; N.T: Not Tested. References are Given in Square Brackets

Oxidative insults	ETC dysfunctions	Related diseases	Effect of MitoQ
Lipoperoxidation	Inhibition of complexes I, III, IV [26, 27, 28, 40, 47, 48]	Ischemia-reperfusion damage [26, 27, 28]	+ [63]
	Impaired cytochrome *b* reduction [46, 47]	Friedreich's ataxia [88, 89]	+ [64]
	Ubiquinone loss [46]	Parkinson´s disease [87]	- [67]
	ROS production [47]	Alzheimer's disease [37]	N.T.
	Cardiolipin peroxidation [26, 27, 28, 38]	Hepatitis C [86]	+ [84]
Thiol oxidation or glutathione depletion	Inhibition of complexes I, II, III [30, 48, 50, 52].	Parkinson´s disease [69]	- [67]
		Hepatitis C [30]	+ [84]
Iron overload	Inhibition of all ETC complexes [38, 40, 43, 46]	Friedreich's ataxia [65]	+ [64]
	Cyt *c* depletion [47]	Hepatitis C [39]	+ [84]
	Ubiquinol oxidation [76]	Alzheimer's disease [36, 37]	N.T.
		Parkinson's disease [33, 34, 35]	- [67]

1). Experiments in brain mitochondria have shown that iron exposure causes diminished membrane potential, impaired ETC function and lipid and protein oxidation. The pre-incubation with an anti-lipoperoxidative agent prevented all these effects except the inhibition of ETC, suggesting that other process besides cardiolipin peroxidation may also be involved in damage to ETC enzymes [38]. Iron overload is also a feature of liver diseases, such as hepatitis C, alcoholic liver disease, porphyria cutanea tarda, or is secondary to systemic diseases, including hemolysis and ineffective erythropoiesis, among others [39]. Hepatic iron overload can severely affect the mitochondrial ETC function, as demonstrated by a study in which a chronic administration of iron inhibited the activity of all ETC complexes, with complex II-complex III being the most sensitive enzymes to ROS damage (Table **1**). These effects were accompanied by an accumulation of iron in the liver, an increment up to 9-fold in the level of free-chelatable iron (which is not surprising, given the role of mitochondria in heme and Fe-S cluster synthesis, as mentioned above), and decrements in both mitochondrial membrane potential and cellular ATP levels [40]. These findings suggest that the mitochondrial ETC can be a key target of the oxidative stress generated during hepatic diseases associated with iron overload.

3. LIPOPEROXIDATION, THIOL OXIDATION AND IRON IN ETC INHIBITION.

Lipoperoxidation and oxidation of sulfhydryl group (-SH) from respiratory enzymes have been identified as the primary factors responsible for impaired ETC activity during *in vitro* oxidative stress or under pathological conditions. Lipoperoxidation occurs in the polyunsaturated hydrocarbon chain of lipids and is started by the abstraction of a hydrogen atom from methylene groups by strong oxidant species like $OH^{\bullet}$ radical, while $O_2^{\bullet-}$ and H_2O_2 alone cannot initiate this process unless $O_2^{\bullet-}$ is converted into its protonated form [41]. Lipoperoxidation yields lipoperoxides and reactive aldehydes, such as malondialdehyde or 4-hydroxynonenal, which inactivate enzymes embedded in biological membranes and decrease membrane fluidity [42]. Thus, lipoperoxidation is a very deleterious phenomenon for mitochondrial function because all ETC complexes, except complex II, depend on the phospholipid cardiolipin for proper structure and function [43].

Three-dimensional X-ray structures obtained from complex III have contributed to a better understanding of the importance of protein-lipid interactions in the enzymatic function of ETC complexes. One of these interactions is exemplified by the binding of phosphatidylinositol to a position near the flexible linker region of the iron-sulfur protein (ISP) in complex III, whose likely function is to allow the rotation of the ISP head domain for electron transfer between the Q_O site and the cytochrome c_1 subunit [44]. Other evidence regarding the dependence of ETC proteins on lipids is the finding that cardiolipin can revert the impairment in the binding of antimycin A to the quinol reductase site (Q_i) done by delipidation of complex III, which reflects a requirement of cytochrome *b* for this lipid to achieve a proper structure [45].

Several papers have reported that the underlying causes of ETC inhibition by lipoperoxidation are cardiolipin and ubiquinone depletion, which affect the enzymatic activities of the complexes and the electron transfer between complexes I and II to complex III [26, 27, 28, 38, 46] (Fig. (**1**), Table **1**). However, in a study using yeast mitochondria exposed to Fe^{2+} (Table **1**) [47], it was suggested that lipoperoxidation can also mediates the impairment of the ETC through the damage in the redox prosthetic groups involved in electron transfer. In this case, the lipoperoxidative damage altered the transfer of electrons to cytochrome *b* and the pre-incubation with the antilipoperoxidative agent butylated hydroxytoluene (BHT) prevented this effect and the inhibition of the activities of complexes III and IV. Furthermore, the role of lipoperoxidation in the alterations in cytochrome *b* was confirmed because BHT also prevented from an increased resistance to the inhibitory effects of antimycin A (an inhibitor that binds to cytochrome *b*) on complex III activity.

In the same study, it was also detected a decrease in the content of cytochrome *c*, which was not associated to lipoperoxidation because this effect was not prevented by BHT or by the innate resistance of yeast to lipoperoxidation; thus, this indicates that the treatment with iron could have mixed effects on some components of the ETC (i.e., lipoperoxidation and protein oxidation) [47]. In agreement with this idea, a mixture of glutathione and lipophilic antioxidants protected the activity of the complex III from rat synoptosomes subjected to iron-ascorbate treatment, suggesting that both lipoperoxidation and –SH oxidation concomitantly impair the activity of the complex [48].

On the other hand, ROS oxidize –SH groups yielding different protein -SH alterations, including formation of mixed disulfides or internal disulfides from vicinal dithiols, S-nitrosation, and the formation of higher oxidation states, such as sulfinic or sulfonic acids [49]. Functional and proteomic studies have detected the presence of free, reactive protein -SH residues [50, 51] that modulates the enzymatic activities of all respiratory complexes, except complex IV (Fig. (**1**), Table **1**) [48, 52, 53].

Regarding the physiological function of –SH oxidation and the modulation of ETC function, it has been postulated that –SH oxidation may protect complex I from oxidative impairment by the following mechanism: complex I activity is oxidatively inhibited by ROS without promoting further ROS generation by selective glutathionylation of only two of six free cysteine residues exposed on the surface of the protein (Cys-535 and Cys-704). Glutathionylation of these –SH may be reverted by reduced glutathione in a reaction catalyzed by mitochondrial glutaredoxin Grx2 to regenerate thiols, allowing the reactivation of the complex and protecting other amino acid residues from oxidative damage [54]. It could be hypothesized that this system operates optimally under high reduced glutathione:glutathione disulfide ratios. Otherwise, -SH would be irreversibly modified to further oxidized forms, leading to irreversibly damaged enzyme and further ROS formation. The importance of glutathione in preventing both an irreversible damage in complex I and an increase in ROS production, can be exemplified by the inhibition of this enzyme observed during hepatitis C infection, in which, the depletion of the mitochondrial pool of glutathione was related to both a decrease in complex I activity and augmented ROS generation (Table **1**) [30].

Although also the complexes II and III have –SH groups whose oxidation inhibits their enzymatic activities in a reversible way [48, 52], their exact role in mitochondrial function remains unclear. Indeed, in some cases, the inhibition of ETC by –SH oxidants like H_2O_2, proceeds by inhibition of enzymes from the Krebs cycle like α-ketoglutarate dehydrogenase and aconitase without directly impairing the activity of ETC enzymes [55, 56]. This also could be related to the fact that the reaction with H_2O_2 does not proceed at physiologically significant rates with -SH unless the formation of thiolate (S^-) species can be favored [57]. The latter can occur in the matrix due to the high pH value of this compartment (~8.0) and the pKa values of protein -SH of 8–8.5 [50]. Therefore, this exemplifies the fact that the damage to the ETC can be influenced by the particular physicochemical conditions of mitochondrial compartments produced by the electrochemical gradient.

In support to the role of protein-SH in the oxidative defense of mitochondria, a recent report has shown that exposed -SH from cysteine residues in the surface of proteins exceeds by 26-fold the concentration of glutathione in the mitochondrial matrix [58]. Due to their abundance and their increased reactivity because of the high matrix pH, it was proposed that the non-enzymatic reactions of protein -SH with ROS may dominate over those occurring with glutathione, Therefore, the exposed protein -SH may be part of an antioxidant cycle in the matrix and may prevent the damage of other amino acid residues on the proteins in which exposed -SH are located [58].

4. THE USE OF MITOCHONDRIA-TARGETED ANTIOXIDANTS TO MITIGATE THE DAMAGE TO ETC FROM OXIDATIVE STRESS: THE CASE OF MITOQ.

As can be appreciated from the above discussed, the design and choice of an antioxidant to protect the ETC will depend on several variables, including the chemical properties of the ROS generated during the disease, the molecular nature of the damage to the ETC (i.e. lipoperoxidation, -SH oxidation or both), and the influence of the electrochemical gradient across the inner mitochondrial membrane on the reactivity of the antioxidant with both the ROS generated and their target molecules.

Disappointing therapeutic effects have been obtained with the use of antioxidants during diseases where mitochondrial oxidative stress is thought to play an important role. For example, neither vitamin C nor vitamin E reduced the risk of developing type 2 diabetes [59] or cardiovascular disease [60] during randomized trials. This prompted to some research groups to develop some antioxidant compounds targeted to the mitochondria. The approach required to deliver these molecules to this organelle consists in covalently attaching certain antioxidant moieties to the lipophilic cation alkyltriphenylphosphonium, which, along with its delocalized positive charge and its permeability in lipid bilayers, can penetrate into mitochondria due to the large negative value of mitochondrial membrane potential [61]. From this class of molecules, one of the most tested has been the ubiquinol derivate MitoQ.

In vitro studies have demonstrated the efficacy of MitoQ in protecting mitochondrial function against oxidative stress. MitoQ protected the activities of complex I and aconitase from the lipoperoxidation induced by H_2O_2 overproduction and subsequent iron uptake by mitochondria in bovine aortic endothelial cells [62]. The activity of complex I was also protected by MitoQ from the inactivation by ROS attack in an *ex vivo* model of ischemia-reperfusion (Table **1**) [63]. MitoQ can also protect against the cell death induced by mitochondrial iron overload in fibroblasts from patients with Friedreich's ataxia, a disease characterized by both defective expression of frataxin, a mitochondrial protein involved in iron homeostasis [64], and impaired complex I activity (Table **1**) [65].

Although these studies have shown beneficial effects in some physiological parameters during diseases involving oxidative stress by ROS exposure, blockage of ETC fluxes, and iron overload, little has been investigated about the molecular mechanisms by which MitoQ protects the function of all ETC complexes from inactivation by lipoperoxidation or –SH oxidation. This is an important issue to address for MitoQ and other molecules under development, because this information could allow a better selection of mitochondrial-targeted drugs from an increasing battery of molecules to achieve more selective effects on ETC function and diminishing the possibility of adverse effects. For instance, although MitoQ is a good inhibitor of mitochondrial lipoperoxidation, a finding that is corroborated by its protective effects on diseases where lipoperoxidation inhibits ETC, such as Friedreich's ataxia or ischemia-reperfusion damage (Table **1**) [63, 64], MitoQ neither scavenges H_2O_2 nor prevents oxidation of biological -SH by peroxides [66]. Therefore, the effectiveness of MitoQ could be limited in diseases where ETC inhibition is due to the oxidation of -SH from mitochondrial complexes. One example that supports this suggestion is the lack of therapeutic efficacy of MitoQ in Parkinson´s disease progression during a clinical trial (Table **1**) [67]. It has been suggested that the inefficacy of MitoQ in this case can be attributed to possible irreversible death of dopaminergic neurons at the stage in which the disease is diagnosed or that a low brain penetration of MitoQ causes insufficient accumulation in mitochondria to exert a protective effect [68]. Nevertheless, since Parkinson´s disease is a condition where the impairment of ETC enzymes proceeds mainly through -SH oxidation [69], it is possible to suggest that MitoQ does not protect against mitochondrial dysfunction because, as mentioned above, this antioxidant cannot prevent -SH oxidation [66]. Furthermore, the ineffectiveness of MitoQ may also be related to the fact that the inhibition of ETC activity in brain mitochondria is independent of the lipoperoxidation mediated by iron overload [38], the latter phenomenon being important in Parkinson´s disease (Table **1**) [34, 33].

However, it is difficult to imagine a disease where only one type of ROS participates in the deleterious effects on mitochondria because of the fast interconversion of $O_2^{\bullet-}$ into H_2O_2 by MnSOD and iron release by H_2O_2 from cellular pools (Fig. (**1**)) to generate $OH^{\bullet}$ radical through Fenton chemistry. Thus, in diseases where a mixture of ROS participates in ETC inhibition, better therapeutic results may be obtained by the concomitant use of antioxidants with different affinity for different molecular targets. For example, -SH oxidation and H_2O_2 production could be mitigated through the incremental adjustment in the mitochondrial levels of reduced glutathione with the use of mitochondria-targeted GSH-choline ester or N-acetylcysteine-choline ester [70], while the lipoperoxidative component of mitochondrial dysfunction could be treated with the concomitant use of MitoQ. Nevertheless, the simultaneous use of mitochondrial-targeted molecules may raise some questions related to the concentrations of the drug required to achieve optimal antioxidative effects without inducing deleterious interactions with ETC complexes. In this regard, MitoQ has been reported to be a redox cycler in association with complex I to produce $O_2^{\bullet-}$ and stimulates apoptosis of endothelial cells [71]. Moreover, the pro-oxidant effects of MitoQ and other mitochondria-targeted antioxidants seem to be concentration-dependent, as demonstrated by a study in which MitoQ was able to inhibit H_2O_2-induced apoptosis at 1 μM, but induces cytotoxicity at concentrations greater than 10 μM [72]. To address the problem of the small ratio between the prooxidant and antioxidant concentrations of MitoQ of less than twofold [73], Skulachev *et al*. have synthesized and tested a new class of triphenylphosphonium derivatives, where the ubiquinone moiety used in MitoQ was replaced by plastoquinone. One of this compounds, SkQ1, has a broad window between prooxidant and antioxidant concentrations of 32-fold [74] and posses a higher antioxidant activity than mitoQ with potential therapeutical effects in the range of low nanomolar concentrations [73].

It has been argued that MitoQ and quinone analogs do not suffer redox cycling under *in vivo* conditions because MitoQ does not modify some mitochondrial markers of oxidative stress and does not alter the mRNA levels of antioxidant defenses when administered orally [68, 75]. Nevertheless, the possibility remains that redox cycling of quinone analogs could not be enough to overcome the *in vivo* antioxidant capacity of cell, so that mitochondria could be able to cope with the quantity of ROS produced by redox cycling without inducing further expression of antioxidant systems. In support to this argument, it must be taken into account that under physiological *in vivo* conditions, the ETC is permanently fed by reducing equivalents from the oxidation of metabolic fuels and it has been shown that a constant feeding of respiratory substrates into the ETC can help to keep the antioxidant capacity of mitochondria at an optimal level, since the energization of mitochondria with respiratory substrates protects mitochondrial membrane lipids from peroxidation by iron/ascorbate, prevents vitamin E depletion and maintains ubiquinone and glutathione in their reduced states [76].

The dependence on membrane potential for mitochondrial accumulation of antioxidants could represent a serious inconvenience in diseases where membrane potential is abolished, as this may favor antioxidant accumulation in other cellular sites different than mitochondria, provoking undesirable effects. For example, the ROS generated by Nox enzymes participate in several processes, such as host defense, synthesis of thyroid hormone, vestibular function in the inner ear, cell differentiation, regulation of the activity of protein tyrosine phosphatase, and other functions [12, 77]. Thus, it is feasible to speculate that if accumulation of the antioxidant is not achieved within mitochondria, the antioxidant may accumulate in other lipophilic environments, such as the cell membrane, where active Nox resides, thus interfering with the functions mediated by Nox enzymes due to scavenging of the ROS produced by this system.

MitoQ may also interfere with processes where the oxidation of biomolecules can has beneficial effects. For example, it has been observed that peroxidative processes in mitochondrial membranes might inhibit tumorigenesis, given that the stimulation of lipoperoxidation through the enrichment of membranes with docosahexaenoic acid (DHA)

induces apoptosis in an immortalized colonocyte cell line; importantly, this process was blocked by the use of MitoQ, but not by the non-targeted antioxidant vitamin E [78]. Therefore, the inhibition of lipoperoxidation by long-term administration of mitochondria-targeted antioxidants may counteract the anti-tumorigenic effects of polyunsaturated fatty acids. In addition, another concern regarding the use of high concentrations of these molecules is mitochondrial membrane depolarization due to the accumulation of large amounts of antioxidant in the matrix, which causes the uptake of these antioxidants to become self-limiting at concentrations above 50 μM [79]. As mentioned above, this issue could be surpassed by the use of a plastoquinone moiety instead ubiquinone, as SkQ1 can cross faster the phospolipid bilayer and is more hydrophobic than MitoQ [74, 80]. Thus, it can be postulated that these properties allow that SkQ1 can serve as an antioxidant in mitochondria at concentrations that not cause severe membrane depolarization.

With respect to the pharmacokinetics of MitoQ, a study has shown that this compound is distributed in heart, brain, liver, and muscle tissues after oral administration [61]. However, it has been suggested that the distribution of these antioxidants to the brain is restricted and that their utility in neuroprotection is questionable [81], which is in agreement with the fact that the vitamin E-derived alkyltriphenylphosphonium cation MitoVit E and MitoQ failed to protect rat neurons from acute hypoxia–ischemia injury [82] and did not exert a protective effect in Parkinson´s disease progression [67]. Four metabolites of MitoQ have been detected after a single oral dose: hydroxylated MitoQ, desmethyl MitoQ and the glucuronide and sulfate conjugates of the quinol form of MitoQ [83]. These data suggest that MitoQ undergoes extensive biotransformation in the liver and, given that these experiments were done in healthy animals, it is very important to evaluate its pharmacokinetic parameters in hepatic diseases to assess the possibility that biotransformation and elimination of this compound could enhance hepatic impairment. In this regard, MitoQ has been subjected to clinical phase II studies during 28-day administration in patients with hepatitis C virus infection, showing benefits in necroinflammation without serious adverse effects [84]. Nevertheless, further long-term studies in liver diseases are required to discard such undesirable effects.

5. CONCLUDING REMARKS

Mitochondrial ETC damage by ROS overproduction seems to be the underlying cause of several diverse pathologies, as ETC dysfunction may trigger upstream signaling and processes that lead to uncontrolled cell death, inflammation, cell cycle arrest, disrupted ion homeostasis and other dysfunctions involved in disease. Therefore, the protection of the ETC from oxidative insults by highly selective mitochondria-targeted antioxidants constitutes a promising therapeutic strategy with relatively few adverse effects. Although we are far from achieving such high selectivity, this issue can be addressed with deeper investigations about the specific ROS participating in disease and studying the structure-activity relationships of ETC enzymes. Such studies will yield more information about which structures of the ETC are more prone to oxidative damage and their roles on impaired electron transfer and ROS production. Finally, it must be stressed that other mitochondria-targeted antioxidants, such as Szeto-Schiller peptides, Skulachev ions or glutathione-choline esters, are currently under study, but it is beyond of the scope of this paper to make a review of all these molecules. Excellent reviews supporting pharmacological strategies targeted to the mitochondria have been authored by Szeto [81], Armstrong [85], Skulachev *et al*. [73] and Camara *et al*. [29] during the last five years.

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